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Multiple Graft Harvestings from Deep Partial-thickness Scald Wounds Healed under the Influence of Weak Direct Current

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The time required for wound healing, contraction, and hypertrophic scarring often limit the use of deep partial-thickness burn wounds as donor sites for split-thickness grafts. We have examined the effects of weak direct current and silver nylon dressings on the healing of partial-thickness scald burns, split-thickness grafts taken from these wounds when healed, and the resulting donor sites in a guinea pig model. Dorsal scald wounds treated with weak direct current reepithelized by 12 days postinjury. Split-thickness grafts taken from healed scald wounds showed more rapid revascularization with direct current treatment than did control grafts. Grafts and donor sites treated with direct current showed more rapid reepithelization, decreased contraction, improved hair survival, and decreased dermal fibrosis when compared to controls not treated with direct current. Only donor wounds treated with weak direct current were reusable as donor sites.

The closure of skin defects by autografting was an historic advance in the treatment of burns. In patients with major burns, however, the burned area is commonly larger than the unburned surface and closure of the wounds with autograft necessitates a staged series of operations in which available donor sites must be repeatedly harvested. The healing time of these donor sites is a major factor in prolonging hospitalization. We have examined the effects of direct current applied through silver nylon dressings (1) on the healing of experimental partial-thickness scald wounds, split-thickness grafts taken from these wounds after healing, and the resulting donor sites.

MATERIALS AND METHODS

Silver Nylon Cloth. Silver nylon cloth (SN) (Swift Textile Metalizing Corporation, Hartford, CT) is a knit nylon fabric, which can be varied from a light weave to a heavy mesh fabric, that is coated with metallic silver to achieve a very conductive, yet flexible material (2). Based on preliminary in vitro studies, Style A-2589-5, a heavy ripstop fabric, was selected for in vivo studies. The material weighed 84.8 gm/m^2 and contained 22.6 gm/m^2 of silver. All SN dressings measured 6.5 × 10.5 cm.

Animals. Two hundred twenty male Hartley guinea pigs weighing 400 ± 25 gm were anesthetized with sodium pentobarbital (35 mg/kg IP). The dorsal trunk hair was clipped and a depilatory cream (Nair™, Carter Products, New York, NY) was applied for 15 min. Partial-thickness scald wounds were inflicted by a 10-sec exposure of the depilated areas to 78°C water using a Walker-Mason burn template with a window measuring 5.5 × 10.5 cm (3). Following scald injury, animals were divided into treatment (n = 180) and control (n = 40) groups as described below. All animals were individually caged in plastic cages insulated from contact with the metal cage stands.

Scald Burn Healing Model. Following scald injury, SN was secured to the wounds with surgical sutures and three layers of gauze and a layer of sponge with a small polyethylene tube attached were placed over the SN dressing. A second contact point was established by adhering a 4 × 5 cm piece of SN to the shaved ventral abdominal skin with electrical-conducting gel. The dorsal SN was connected as the anode in the circuit. The gauze was then fixed in place with a flexible tubular bandage. To prevent the animals from disturbing the electrode wires and irrigation tube, the wires and tube were passed through a hole cut in a wooden tongue blade and a 4-in length of meshed wire insulator. The blade was then sutured to the back over the dressings (Fig. 1). The gauze was moistened daily with 3 to 5 ml of saline through the irrigation tube. A constant 40 μA direct current (DC) was applied for 2 days, followed by 20 μA for 3 days, using a previously described constant DC generator (4). Scald wounds in the control group were dressed as above, but no DC was applied. No dressings were applied after the fifth postburn day. Gross and microscopic comparisons of wound healing in ten treatment animals and two control

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The care of all animals was in accordance with the guidelines set forth by the Animal Welfare Act and other Federal statutes and regulations relating to animals and studies involving animals and with the *Guide for the Care and Use of Laboratory Animals*, National Institutes of Health Publication 86-23.

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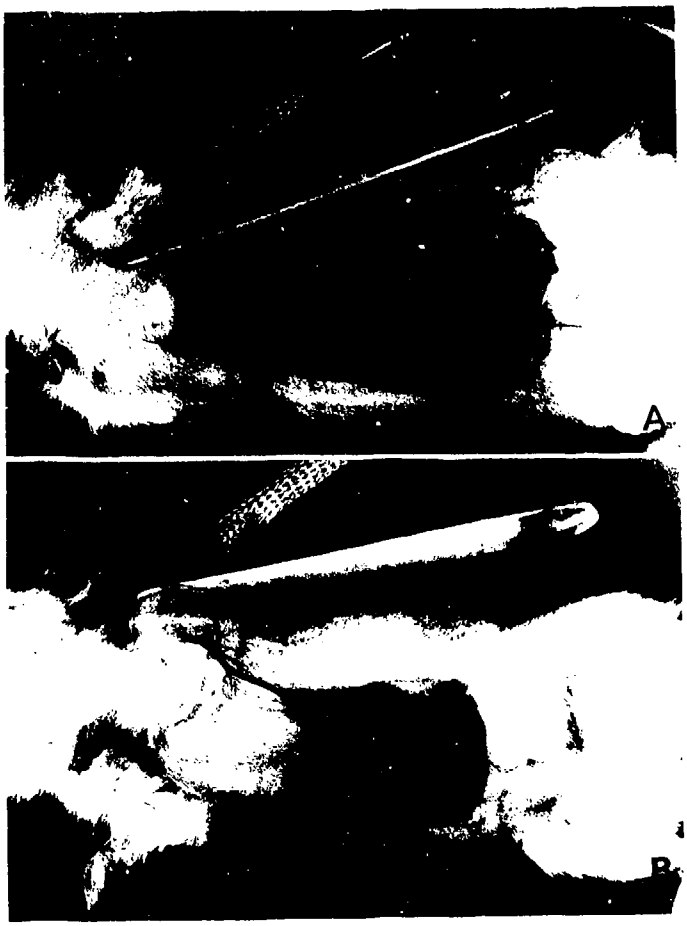

FIG. 1. Locations of SN anode and cathode on scalded animal. **A)** The dorsal dressing is sutured over the wound and connected to DC as an anode. **B)** SN cloth coated on the underside with conductive gel is stapled to the abdomen and connected to DC as a cathode.

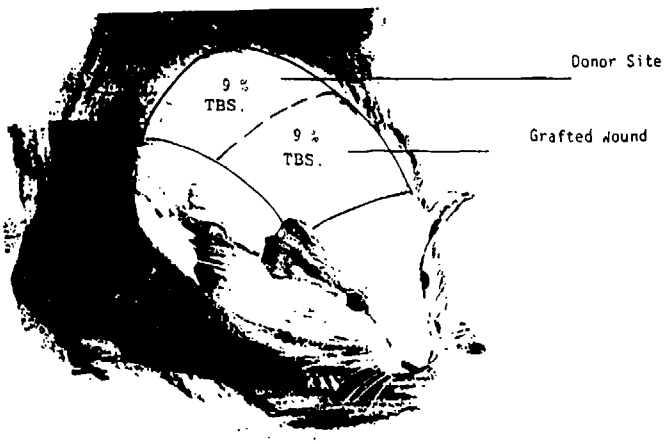

FIG. 2. Diagram of scalded wound area and location of donor site and grafted wound.

animals were made at 2, 3, 4, 7, and 14 days and 3 months postburn. The depth microcirculation in the wounds of animals sacrificed at 2, 3, 4, and 7 days postburn was estimated by perfusion via a cannula placed in the superior mesenteric artery with black ink (Drawing Ink A, Pelikan AG, D-3000, Hanover, West Germany). All biopsy specimens were taken from the central area of each site, and estimation of the extent of microcirculation in the wounds was made by microscopic examination for the presence of carbon particles in the vessels.

Wound Excision and Grafting Model. When the scald wounds reepithelialized (see below) in 120 animals randomized to the treatment group and 20 animals randomized to the control group, 0.015-in split-thickness grafts were taken using a Padgett™ electrodermatome. Each graft was divided in half and the anterior portion of the graft bed was covered with the posterior half of the graft. The anterior portion of the graft was discarded. Each animal thus had an anterior autografted wound segment and an open posterior donor site segment of equal size. A diagram of the model is presented in Figure 2. Animals were then dressed as above with those in the treatment group receiving 40 μA for 2 days followed by 20 μA for 3 days. Graft and donor sites were either biopsied at the time of sacrifice for microscopic examination or visually examined daily for 3 weeks and then weekly. The extents of reepithelialization, hair growth, and gross wound contraction were recorded. Samples of graft and donor site tissues were obtained and examined microscopically in ten treatment animals and two control animals for anatomic evidence of scarring and depth of microcirculation on the day of harvesting and 2, 3, 4, 7, and 12 days after excision and grafting.

Multiple Harvesting Model. The remaining 60 animals in the excised, grafted, and electrically treated group reepithelialized both donor sites and grafted areas by 14 days after the harvesting. The entire area (0.015 in) of the original scald was again harvested and the graft from the healed donor site was placed on the anterior portion of the bed. The posterior area was again not grafted and left as an open donor site. Animals were again dressed and treated as had been previously done. Second grafts and donor sites were examined as above.

Estimation of Hair Follicle Survival. Fourteen days after the last treatment in each group, hair follicles were counted in the histologic sections. Sections from ten individual animals in each group were examined at a magnification of 100×. Three random fields of the upper half and the lower half of the dermis were counted. Comparison of follicle counts were made by *t*-testing of treatment and control results.

RESULTS

Scald Wound Healing Model. By 12 days postburn, all animals in the treatment group had completely resurfaced wounds. In contrast, only 50% of the animals in the control group had reepithelialized wounds by 16 days postburn. Examples of treated and control wound healing are shown in Figure 3. Figure 3A shows the wound appearance of treated animals at 12 days postburn. Microscopic examination (Fig. 3B) showed that these treated animals had minimal dermal loss and little subepidermal granulation tissue. Most of the hair follicles had survived. Reepithelialized control wounds remained partially covered with a thick layer of dried eschar at 16 days (Fig. 3C). Microscopic examination showed that approximately 30% of the original dermis has been replaced by a layer of granulation tissue (Fig. 3D) and surviving hair follicles were rare. When present, these follicles originated in the deeper dermis.

The animals in the control group that had reepithelized wounds by 16 days postburn were used as donors of split-thickness autografts. The control scald wounds not healed at 16 days required several additional weeks for complete healing and could not be used for subsequent excision and grafting studies.

Graft Healing Model. Autografts taken from the

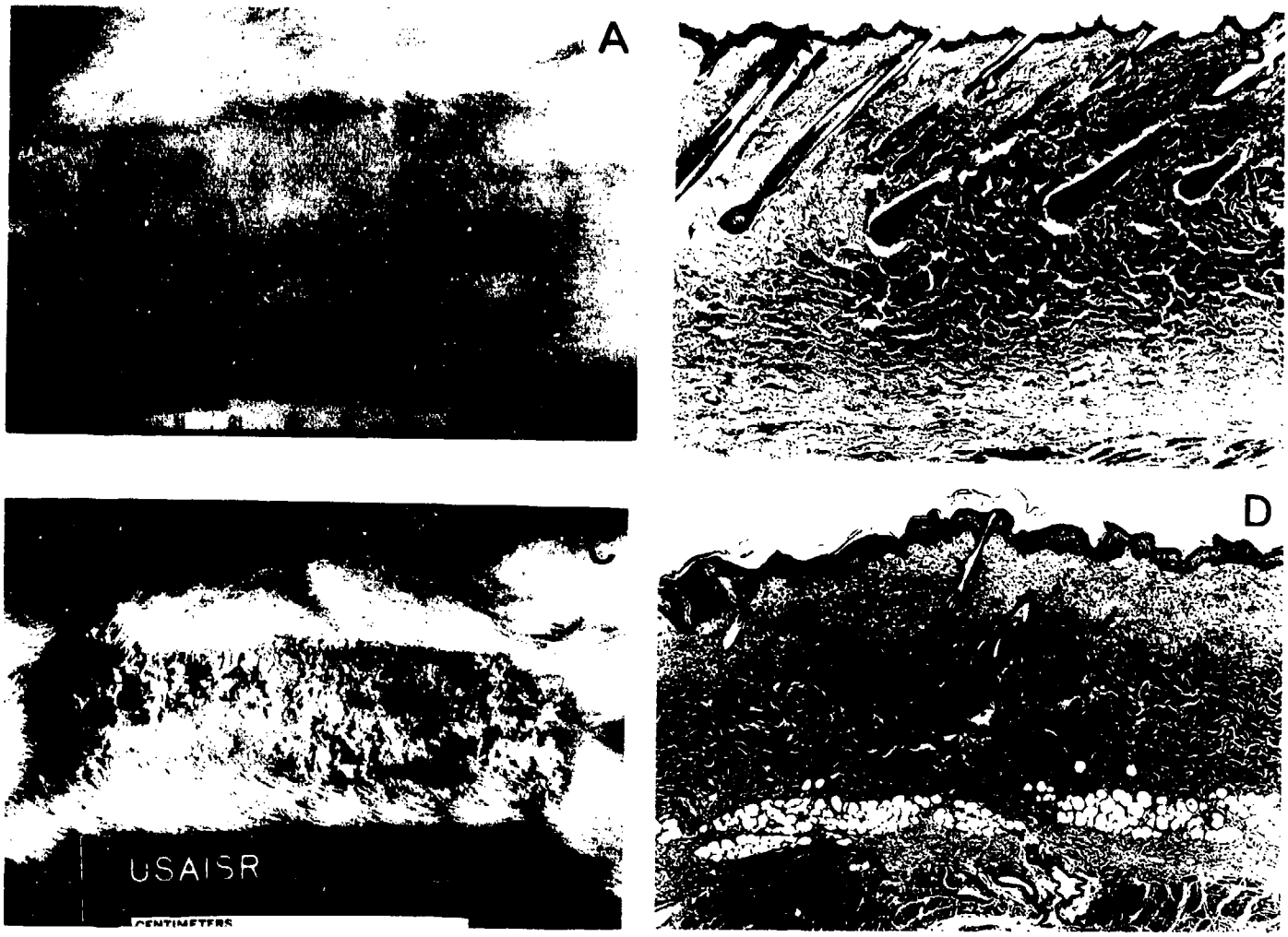

FIG. 3. Comparison of treatment and control animals. **A)** Gross appearance of a treated scald wound 12 days after wounding. The wound is completely reepithelialized. **B)** Microscopic appearance of the wound in Panel A (trichrome, 10×). The wound is reepithelized and contains numerous hair follicles. **C)** Gross appearance of a control group scald wound that reepithelialized 16 days after wounding. There is a dried crust containing epithelial debris on the wound surface. **D)** Microscopic appearance of the wound in Panel C (trichrome, 10×). Even in an area of healing, there is subepidermal inflammatory infiltrate and loss of hair follicles in the healed wound (arrow).

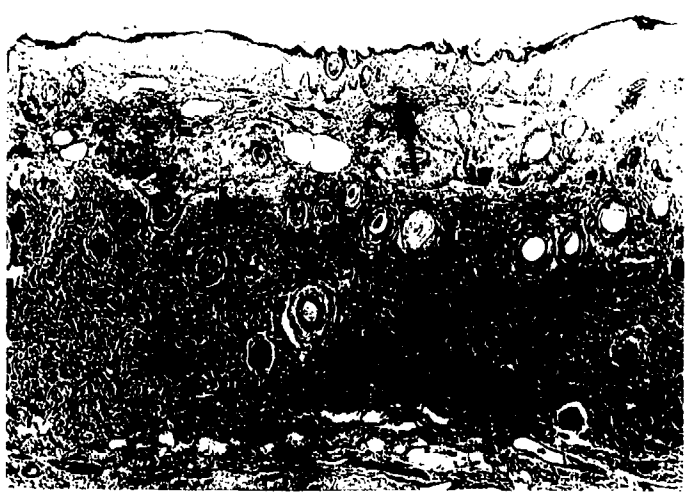

FIG. 4. Grafted wound from a treated animal 4 days after harvesting from a healed scald wound (H&E, 10×). Carbon from black ink is evident in grafted tissue (arrow). Hyperplasia in hair follicle epithelium is present at graft-wound interface and gives the appearance of an epithelial layer joining the graft with the wound bed (arrowhead).

reepithelialized scald wounds of treated animals were firmly adherent 4 days after grafting. Microscopic examination at this time showed the presence of ink carbon in the grafted tissue (Fig. 4), indicating that union between graft and wound microcirculation had been established. In addition, there was an unusual hyperplasia of hair follicle epithelium at the graft-wound interface. This hyperplasia was most obvious at 4 days after grafting when it gave the appearance of an epithelial layer joining the graft with the wound bed. Figure 5 shows that by 7 days after grafting, hyperplasia of hair follicle epithelium at the graft-wound interface was less prominent and nearly normal hair follicles had reformed. At 12 days after grafting, a stratum corneum was evident and the architecture was that of normal guinea pig skin. Second grafts taken 14 days after primary harvesting in treated animals and again treated with DC showed gross and microscopic findings essentially similar to those described for primary grafts.

Autografts from reepithelialized control scald wounds showed first evidence of revascularization and weak ad-

FIG. 5. Grafted wound from a treated animal 7 days after harvesting from a healed scald wound (H&E, 10×). In this longitudinal section, the hyperplasia of hair follicle epithelium at the graft-wound interface is less prominent and nearly normal hair follicles have reformed (arrow).

FIG. 6. Revascularization of a grafted wound from a control animal 7 days after harvesting (H&E, 10×). Black ink carbon is present in the graft tissue. Most of the hair follicles have degenerated. The inset of the superficial dermis and epithelium shows ink particles in the vessels (arrow) and cords of the hyperplastic epithelium (arrowhead) (H&E 40×).

herence 7 days after grafting. This was 5 full days later than in treated animals. As shown in Figure 6, further degeneration of the hair follicles had occurred by 1 week after grafting. Cords of hyperplasic epithelium extended down from the epidermis. By 3 months after harvesting, graft contraction and hair loss were marked in these animals.

Donor Site Healing Model. By 48 hours after the first harvest and DC treatment, epithelial cells in the donor site hair follicles showed evidence of initial surface migration and no significant inflammatory reaction was seen in ten of ten examined animals. Samples taken at 3 days showed donor wounds covered with a neoepidermis of 3- to 8-cell thickness in ten of ten treated animals examined, but stratum corneum was not obvious. As shown in Figure 7A, treated animals at 14 days after harvesting had healed grafted wound and donor sites. Wound contraction was not obvious. Figure 7B shows that these donor sites had an established stratum corneum and an essentially normal distribution of the hair follicles. There was moderate acanthosis of the epidermis and minimal fibroblast growth in the subepidermis in some areas. No gross contraction was evident 2 weeks or 3 months after harvesting and both graft and donor sites expanded with growth of the animal. Hair density was only slightly reduced.

Donor site healing after the second harvesting in treated animals required several days longer for reepithelialization and stratum corneum formation than did the primary donor sites. Donor sites had an established stratum corneum and survival of most hair follicles at 16 days after the second harvesting. Figure 8A shows the appearance of treated animals at 16 days after the second harvesting of a donor site. As shown in Figure 8B, minimal subepidermal fibroblast proliferation was present in the donor site. At 3 months, the graft and donor sites showed minimal contraction and hair loss (Fig. 9). Although further graft harvesting from healed, twice harvested donor sites was not attempted, there was no gross or microscopic evidence to suggest that such grafts could not be successfully taken.

Donor sites of control animals showed neutrophil accumulation at the wound surface 48 hours after an initial graft harvest. At 3 days, epithelial cell migration from the remaining hair follicles had initially advanced under a thin layer of inflammatory cells. Reepithelialization of donor wounds after harvesting in control animals required more than 3 weeks. As shown in Figure 10, donor site wounds had marked fibrosis in the upper dermis and most hair follicles had degenerated in the reepithelized areas at 3 weeks after harvesting. Contraction was

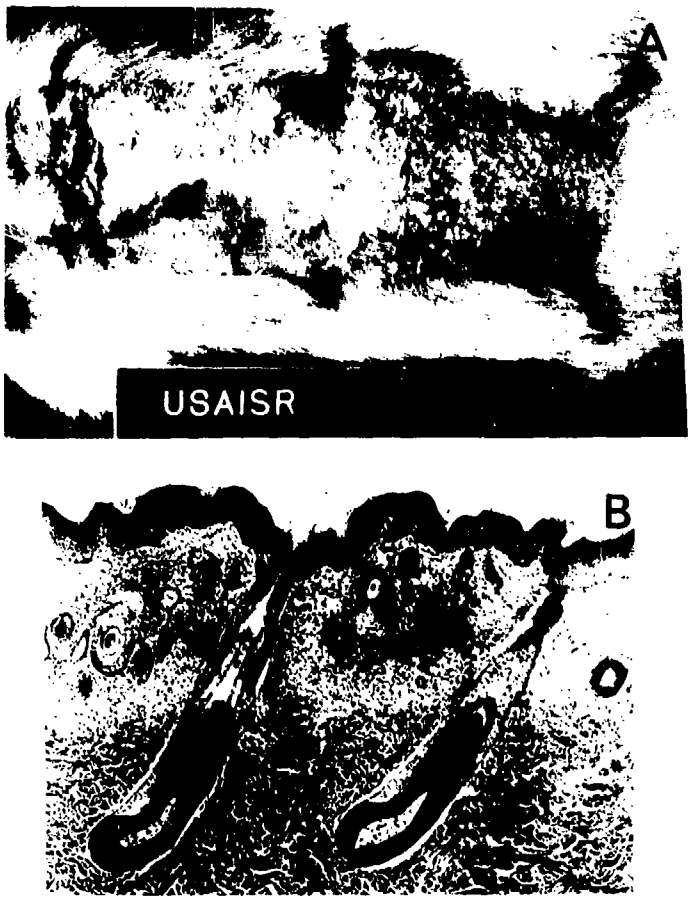

FIG. 7. Previously healed scald wound of a treated animal 14 days after harvesting and grafting. **A)** Gross appearance shows healed graft wound and donor site. **B)** Microscopic appearance shows survival of many hair follicles and essentially normal epidermis with moderate acanthosis (H&E, 10×).

marked and no further harvesting of grafts from control animals was attempted.

DISCUSSION

The original hypothesis that DC would improve burn wound healing was based on observations of healed wounds of animals surviving after treatment of experimental burn wound sepsis with silver nylon anodal dressings (1). The results of the present study, designed to examine this possibility using partial-thickness scalds, clearly show that DC markedly reduces the time required for healing and improves the quality of the healed wounds. Although the anatomic evidence is obvious, the strongest argument is that treated scald wounds could be repeatedly harvested as donor sites for highly successful split-thickness autografts.

Although the mechanisms of the improvement in healing were not addressed in this study, the results lead us to hypothesize that the observed effects may be due to the prevention of deleterious events that occur in the wound following injury and cause progression from cell injury to cell death. DC treatment may not cause improvement in wound healing but may instead limit the extent of tissue destruction. In all treated groups, the

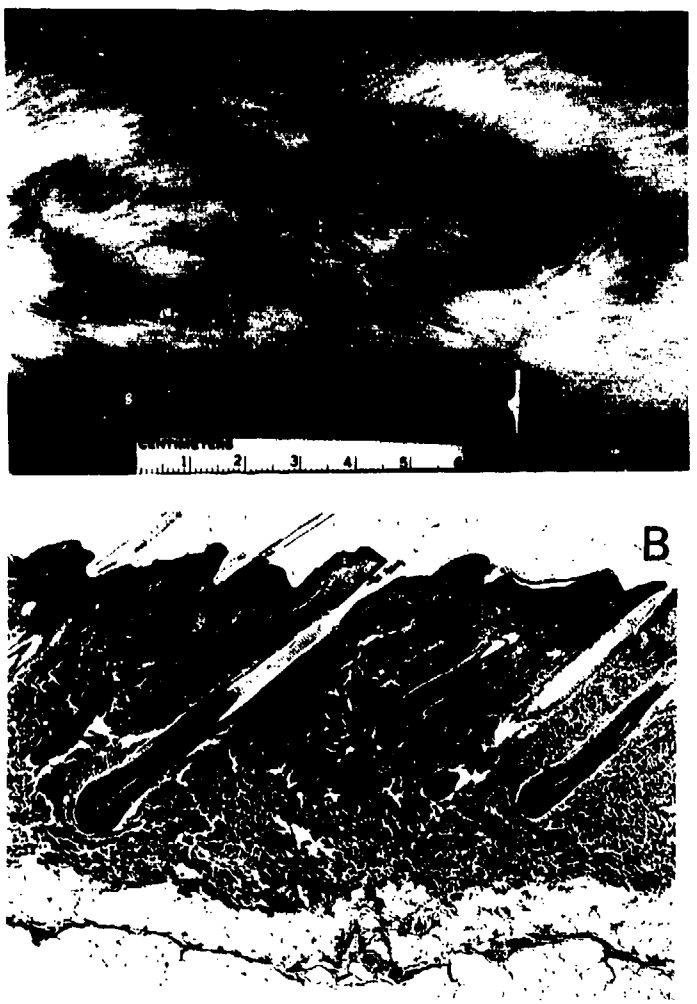

FIG. 8. Scald wound of a treated animal 16 days after the second split-thickness skin graft harvesting. **A)** Gross appearance shows no obvious contraction. **B)** Microscopic examination of the donor site reveals only minimal evidence of subepidermal fibrosis and little inflammatory cell infiltration (H&E, 10×).

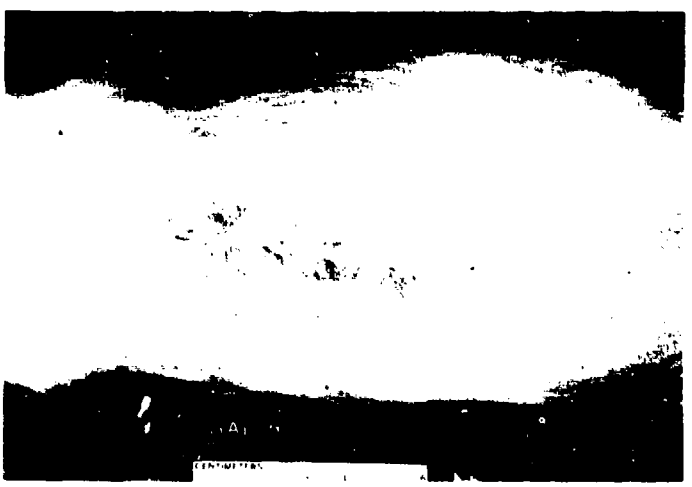

FIG. 9. Scald wound of a treated animal at 3 months after the second harvesting. There is minimal contraction and hair loss.

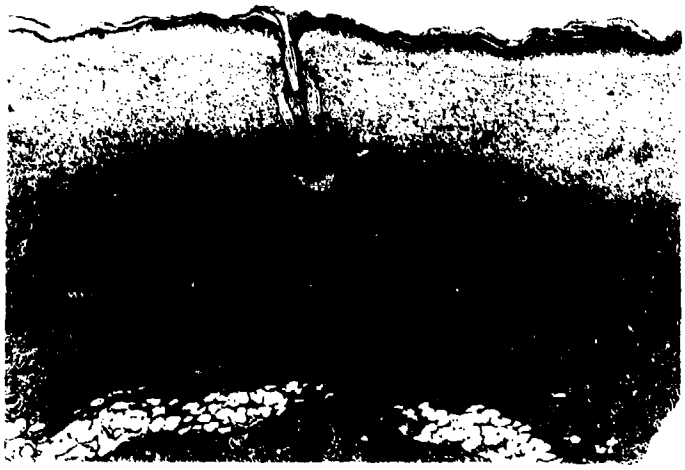

FIG. 10. Microscopic appearance of the donor site from a control animal 21 days after harvesting and grafting from a healed scald wound (trichrome, 10×). The donor site shows subepidermal fibrosis (arrow) and loss of hair follicles.

wounds showed less inflammation, granulation tissue, and fibrosis than in the control groups. The observed shortening in reepithelialization time may simply be a consequence of a much greater number of surviving active hair follicles. As shown in Table I, the improved survival of hair follicles with DC treatment was readily demonstrated by microscopic counts of hair follicles in the upper and lower halves of the dermis of all groups. The active hair follicles of treated animals allowed healing to occur predominantly from within the partial-thickness wounds and donor sites themselves, rather than from the wound edges as occurred in control animals with few residual hair follicles within the wound. The mechanism for this proposed prevention of progression of injury could be relief of circulatory stasis and ischemia in the wound margins (5). If limitation of blood flow and depletion of required metabolic factors causing cell death are secondary effects of injury which evoke inflammation and influence other stages of wound healing, any reduction in such tissue loss would be expected to result in reduced inflammation and, in turn, less scarring and wound contraction. The microscopic findings in this study, i.e., less inflammation and less fibrosis in treated animals, are fully consistent with this speculation. Such a mechanism might also explain improved graft and donor site healing with DC treatment, since such wounds also experience at least transient periods of ischemia. The more rapid reestablishment of the microcirculation in the wounds and grafts of the animals treated with direct current may account for the reported observations.

Acknowledgment

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DISCUSSION

DR. THOMAS K. HUNT (San Francisco, California): Thank you. Under the influence of new data on growth factors and oxygenation/perfusion of wounds, there has developed a new willingness to look at the possibility that wound healing can be manipulated.

In this light we might be surprised but not astounded or offended by these revelations about dramatic changes in healing of partial-thickness scalds and donor sites in guinea pigs by what I have termed for today TLC, or topical 'lectrical care.

Electricity has had special attraction for wound healers for many years, and there are many studies in the literature, most of them contradicting the last about alternating currents, direct currents, square waves, voltages, amperages, et cetera.

The net result is conviction that electricity does something to repair, but we don't know what. This is natural and acceptable since chemical and hormonal signals eventually are transmitted by flow of electrons and since wounds have electrical polarity.

The present study, however, is unusual in two respects. First, the approach is topical, so to speak, diffused onto the surface.

TABLE I
Hair follicle survival in healed wounds (mean counts/field)

	Scald Wound		First Use Donor Site		Second Use Donor Site
	Treatment Group	Control Group*	Treatment Group	Control Group*	Treatment Group
Upper dermis	8.8 ± 0.8	3.8 ± 0.7†	6.6 ± 1.0	3.8 ± 0.4†	3.8 ± 0.6
Lower dermis	6.4 ± 0.4	2.0 ± 0.7†	5.6 ± 0.9	1.2 ± 0.3†	3.7 ± 0.4

* Tissue specimens obtained from the epithelized portions of wounds and donor sites.

† $p < 0.02$.

Surviving hair follicles were counted at 14 days after scalding and after first and second harvestings. Groups were compared by *t*-test and data are presented as means of 30 random fields examined in 10 animals per group.

Most others have used current polarized at right angles to full-thickness wounds.

It is also unusual in that electricity was applied immediately to surface burn wounds which characteristically deepen by progressive vascular necrosis over a period of days. The data appear to suggest that the weak constant current applied to these wounds limits the progressive necrosis.

I know of only one previous similar study, in which "topical" electricity was used to induce regeneration of forefeet in rodents after amputation, an effect which, of course, is not shared by humans. From this study we derive some cautions about interpreting the present data. Whether this effect can be extended to humans is, of course, in doubt and will be determined only by human testing. Second, what are the time limitations? When must this treatment be started in order to have its effect? Does it lose its effect after 2 or 4 or 48 hours?

I would also like to ask about the polarity of the current. Was it the positive or negative pole that was on the burn? That has made a difference in bone experiments.

Fortunately, there are few serious obstacles to carrying on this kind of research in man, and if this can reach the spectacular effect in man that it has in guinea pigs, we should know about it quite soon. I am looking forward eagerly to followup studies. Thank you. [Applause]

DR. GLENN WARDEN (Cincinnati, Ohio): I would like to congratulate the authors on an interesting paper. It appears that the major difference between both the healing and the grafted areas was the presence of hair follicles, in that the deep second-degree burns treated with electricity had many more hair follicles present. I wonder if the authors could speculate on this.

In addition, it appears that the recipient sites had hair growth. Would the authors speculate on the depth of these grafts, and was there a difference between the two groups?

DR. WILLIAM MONAFO (St. Louis, Missouri): I enjoyed this very much. We are used to getting lots of numbers from the U.S. Army Institute of Surgical Research. We didn't get the benefit of the numbers but the pictures were most impressive. I would like to ask a couple of questions. One, how did you select the amperage? Why did you select the amperage that you did use? Second, is this a phenomenon that is specific to thermal injury? Have you done this in perhaps a better controlled model, that is, simply cutting the skin graft at a predetermined thickness and then determining its healing time?

I enjoyed this paper very much.

DR. ALBERT MCMANUS (Closing): I would like to thank you very much.

I honestly didn't expect to be up here. [Laughter] To speak to Doctor Hunt's question. The anodal polarity is necessary at the wound surface. I will go on to explain to you why we think that is true.

The energies involved in this thing may not be obvious to you. We are talking about over that entire surface, 40 microamps, and the maximum voltage was 1 volt. So we are talking about very low energies here, essentially less than commonly used with the muscle relaxant things that people are running around with these days.

In fact, that is what we are looking at now to try to avoid electrical questions across the chest. We are planning on using already approved electrical supplies to drive this device in man hopefully within the very near future.

As far as delay of the time for effect, we really don't know that. The original observation was based on early results with silver nylon cloth being an antimicrobial agent where the silver content could be controlled by the amperage applied, and in those experiments the surviving animals had noticeably different wound quality and those were not applied for 24 hours. But they were full-thickness injuries so we are talking about edge changes rather than at the center of the graft.

I don't know how much time we have to observe this difference.

As far as Doctor Warden's questions, this again goes back to the original observations of this being an antimicrobial device, and the wound healing aspects of it came out of that. The dermal depth of the hair follicles is actually what Doctor Chu was trying to bring across. What he is doing is as much preventing the wound from forming as to improve wound healing. What you are seeing is the survival of much more of the dermis in the treated animals than you do in the control. Control wounds without electricity result in an increase in scarring and a 30% decrease in dermal thickness that you can see see in the wound. That is, what you are doing is saving that dermis with the treatment.

With the excised wounds, the donor site and the excised bed both have viable hair follicles. In fact, the slide that Doctor Chu didn't show you that will be in his paper is a very interesting phenomenon. Early on in the healing process he has two layers of epithelial growth. He has the epithelium growing up from the original wound and from the terminal has part of the follicles in the graft plus the graft, the upper epithelium. Actually, it has two layers of epithelium that we would normally think would result in a slough, but it does not. The lower levels of epithelium either degenerate in place or find their way and reconnect with existing hair follicles.

I do not know of any example of that in the literature. It is really strange, but it is there.

Doctor Monafo's questions about another model: yes, in fact, Doctor Chu has done this in two graft situations, unburned animals with grafts, the most interesting of which are full-thickness graft flaps to the panniculus muscle. He can reproducibly raise the graft and turn it 180° which is the best proof I know for take and when the animal recovers at 3 months, you have the hair growing the wrong way, so this is definitely something going on very fundamental to wound healing and hopefully we will be able to learn from these models and go on to man.

Thank you. [Applause]